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13. ABSTRACT (Maximum 200 Words)

The cell lines derived from BRCA2 mutant mice (complete knockout and C-terminus trancated mutant) are hypersensitive to the DNA crosslinking agent, mitomycin C (MMC). MMC induces DNA interstand crosslinks (crosslinks), which is repaired b an ill-defined crosslink repair pathway. Our hypothesis is that BRCA2 directly participates in the removal of crosslinks by forming a DNA repair complex with other factors. Our plan is to isolate a BRCA2-crosslink repair complex using our cell-free crosslink repair assay. In order to accomplish this goal, we need to prepare cell free extract from BRCA2 mutant cells. There are currently three sources of BRCA2 mutant cells, MEF cells from BRCA2 mutant mice, CAPAN-1 cells and XRCC-11 Chinese hamster mutant cells. We chose to use CAPAN-1 cell because CAPAN-1 cells are available from ATCC. We examined the sensitivity of CAPAN-1 cells to MMC and UV radiation. Similar to the reported phenotypes or mouse and hamster BRCA2 mutant cells, CAPAN-1 cells are sensitive to MMC, but not to UV irradiation. The data showed that CAPAN-1 cells are most likely defective in crosslink repair. We are currently growing CAPAN-1 cells in a large scale to prepare cell-free extract. Once we prepare cell-free extract, we will proceed with the proposed experiments to isolate a BRCA2-containing crosslink repair complex.

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Introduction

Mutations in tumor suppressor gene BRCA2 result in genomic instability, which is the hallmark of cancer cells (1, 2). Accumulating evidence, mostly from genetic studies, indicates that BRCA2 is one of the key factors in *DNA double strand break (DSB) repair* (3). *BRCA-2* mutant cells are sensitive to γ-ray irradiation and defective in double strand break repair by homologous recombination (4, 5). Furthermore, it has been demonstrated that BRCA2 interacts with a recombination protein RAD51 through the BRC repeats (6, 7) and this interaction modulates DNA binding activity of RAD51 *in vitro* (8, 9). These data clearly show the role of BRCA2 in DSB repair.

Curiously, mutations in BRCA2 also result in the hypersensitivity to DNA crosslinking agents, which generate interstrand DNA crosslinks (crosslinks; 10, 11). Crosslinks are very unique DNA lesions because bases in both strands of the DNA duplex, that are one or two bases away on the opposing strand, are damaged simultaneously and linked covalently. Currently, how these factors function together to remove crosslinks is not well defined. It has been reported that DNA replication-mediated formation of DSB is the intermediate during crosslink repair (12-15). BRCA2 and other DSB repair proteins might be involved in the processing of the intermediate DSB in crosslink repair.

We recently developed a cell-free crosslink repair assay. By adding biochemically fractionated proteins from HeLa cells to cell-free extract prepared from crosslink repair defective BRCA2 mutant cells, we should be able to isolate a BRCA2-containing crosslink repair complex. The identification of BRCA2-associated proteins in the complex will help to understand the molecular basis of the tumor suppressor function of BRCA2.

Body

Before the statement of our accomplishment in the first year of the award, I would like to add an important note. **The award was suspended from June of 2003 to January of 2004 due to the PI's relocation from the University of Texas Health Science Center at San Antonio to the University of Nebraska Medical Center.**

Task 1: Isolation of a crosslink repair complex containing BRCA2 protein.

In order to accomplish Task 1, we need at least 10^9 cells of BRCA2 mutant cells to prepare cell extract for cell-free crosslink assay. Currently, there are three sources of BRCA2 mutant cells, MEF cells from BRCA2 mutant mice, CAPAN-1 cells and *XRCC-11* Chinese hamster mutant cells. Only CAPAN-1 cells are available from ATCC. CAPAN-1 cells are γ-ray sensitive due to the defect in DSB repair. However, the cellular sensitivity of CAPAN-1 cells to DNA crosslinking agents has not been reported, although all other cellular responses to DNA damage are quite similar to those of the MEF cells from BRCA2 mutant mice and *XRCC-11* cells (2, 10, 11). Therefore, we examined the sensitivity of CAPAN-1 cells to MMC. BxPC3 cells were used as a BRCA2 proficient control as has been reported (2). Our preliminary results with a dye exclusion assay showed that CAPAN-1 cells are three to five-fold more sensitive to MMC compared to BxPC3 (Fig. 1). CAPAN-1 cells were not particularly sensitive to UV irradiation as is the case for MEF cells from BRCA2 mutant mice (10). These results demonstrated that CAPAN-1 cells are defective in crosslink repair.

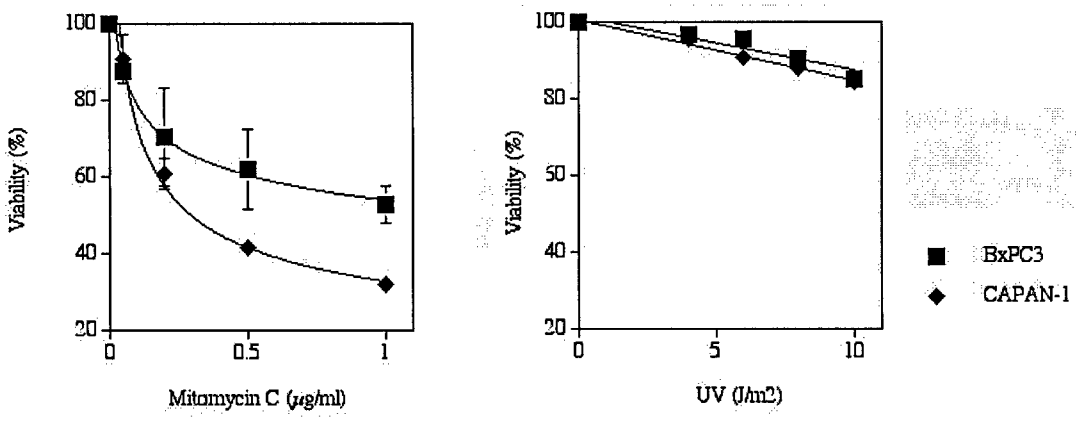

Fig. 1. CAPAN-1 cells are sensitive to Mitomycin C treatment but not to UV irradiation. Cells were seeded into 96-well plates at 4,000 cells/well for BxPC3 and at 10,000 cells /well for CAPAN-1 cells and were grown overnight. The cells were treated with the indicated concentration of MMC for two hours. After removing MMC by washing with PBS twice, the cells were grown for additional three days. For UV experiments, after washing the cells twice with PBS, PBS was removed from the wells, and then the cells were irradiated by UV at the dose of 1 J/m^2/sec. The irradiated cells were grown for additional three days. Cell viability was determined by standard MTT assay. The data for the MMC treatment are the average of five independent experiments and the bars in the graph are the standard deviations (some are not visible in the graph due to very small variations).

We are currently growing enough CAPAN- 1 cells to prepare cell-free extract for our crosslink repair assay. The process has been taking longer than we planned because CAPAN-1 cells grow very slowly with a doubling time of 48 hrs. Once we obtain enough numbers of CAPNA-1 cells, we will prepare cell-free extract and will proceed with the proposed experiments to isolate a BRCA2-containing crosslink repair complex.

Key research accomplishments

As stated above, we found that CAPAN-1 cells are sensitive to DNA crosslinking agent, mitomycin C. The results indicate that the cellular sensitivity specific to mitomycin C is the common feature of all BRCA2 mutant cells. Therefore, a function of BRCA2 in crosslink repair should be one of the critical factors in tumor suppressor function of BRCA2.

Reportable outcomes

There is no reportable outcome yet.

Conclusion

This year, we obtained important data to proceed with our proposal. As we proposed in the original application, CAPAN-1 cells are sensitive to DNA crosslinking agent, mitomycin C. The data is critical to our proposal because the CAPAN-1 cell is the only source of BRCA2 mutant cell-line available from ATCC. We are now in the position to examine the crosslink repair activity in CAPAN-1 cells with cell-free crosslink repair assay and to extend our research to isolate a BRCA2-containing crosslink repair complex.

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Appendices

None